

### **AMENDMENTS TO THE SPECIFICATION**

Please delete the previously submitted Sequence Listing and replace with the Sequence Listing submitted on compact disc enclosed herewith.

In the specification at page 1, after the title, please insert the following new paragraphs:

#### **RELATED APPLICATIONS**

This application is a national stage application (under 35 U.S.C. 371) of PCT/EP03/06994 filed July 1, 2003, which claims benefit of European application 02017671.5 filed August 7, 2002.

#### **SUBMISSION ON COMPACT DISC**

The contents of the following submission on compact discs are incorporated herein by reference in its entirety: two copies of the Sequence Listing (COPY 1 and COPY 2) and a computer readable form copy of the Sequence Listing (CRF COPY), all on compact disc, each containing: file name: Sequence Listing - 13311-00012-US, date recorded: April 30, 2007, size: 114 KB.

#### **BACKGROUND OF THE INVENTION**

In the specification at page 6, line 18, please insert the following new paragraph:

#### **SUMMARY OF THE INVENTION**

In the specification at page 8, after line 11 and before line 12, please insert the following new section:

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1: The glutaredoxin gene family showing the four subfamilies of glutaredoxin and thioredoxin coding sequences as determined by amino acid sequence homology. The abbreviations correspond to the "Gene ID" as explained in Table 6.

Figure 2: Amino acid alignment of yeast and plant cDNA sequences of glutaredoxin subfamily 1 showing the presence of two conserved domains. The abbreviations correspond to the "Gene ID" as explained in Table 6.

Figure 3: Amino acid alignment of glutaredoxin subfamily 1 domain 1 across yeast and plant cDNA sequences (GRX1, SEQ ID NO: 2; GRX2, SEQ ID NO: 4; AtQ9FM49, SEQ ID NO: 26; AtQ9FNE2, SEQ ID NO: 28; AtQ9SK75, SEQ ID NO: 34; BN1106C12219, SEQ ID NO: 16; BN1106C2202, SEQ ID NO: 20; OZ1116C26232, SEQ ID NO: 50; OZ1116C2194, SEQ ID NO: 48). The amino acid position at the start of the alignment is shown in parenthesis.

Figure 4: Amino acid alignment of Glutaredoxin subfamily 1 domain 2 across yeast and plant cDNA sequences (GRX1, SEQ ID NO: 2; GRX2, SEQ ID NO: 4; AtQ9FM49, SEQ ID NO: 26; AtQ9FNE2, SEQ ID NO: 28; AtQ9SK75, SEQ ID NO: 34; BN1106C12219, SEQ ID NO: 16; BN1106C2202, SEQ ID NO: 20; OZ1116C26232, SEQ ID NO: 50; OZ1116C2194, SEQ ID NO: 48). The amino acid position at the start of the alignment is shown in parenthesis.

Figure 5: Amino acid alignments of yeast and plant cDNA sequences of glutaredoxin subfamily 2 showing the presence of two conserved domains. The abbreviations correspond to the "Gene ID" as explained in Table 6.

Figure 6: Amino acid alignment of glutaredoxin subfamily 2 domain 1 across yeast and plant cDNA sequences (THX1, SEQ ID NO: 12; THX2, SEQ ID NO: 14; GRX3, SEQ ID NO: 6; GRX4, SEQ ID NO: 8; BN1106C23043, SEQ ID NO: 24; AtQ65541, SEQ ID NO: 42; AtQ9ZPH2, SEQ ID NO: 44). The amino acid position at the start of the alignment is shown in parenthesis.

Figure 7: Amino acid alignment of Glutaredoxin subfamily 2 domain 2 across yeast and plant cDNA sequences (THX1, SEQ ID NO: 12; THX2, SEQ ID NO: 14; GRX3, SEQ ID NO: 6; GRX4, SEQ ID NO: 8; BN1106C23043, SEQ ID NO: 24; AtQ65541, SEQ ID NO: 42; AtQ9ZPH2, SEQ ID NO: 44). The amino acid position at the start of the alignment is shown in parenthesis.

Figure 8: Amino add alignments of yeast and plant cDNA sequences of glutaredoxin subfamily 3 showing the presence of two conserved domains. The abbreviations correspond to the "Gene ID" as explained in Table 6.

Figure 9: Amino acid alignment of glutaredoxin subfamily 3 domain 1 across yeast and plant cDNA sequences (GRX5, SEQ ID NO: 10; AtQ9SV38, SEQ ID NO: 38; AtO80451, SEQ

ID NO: 40; AtQ9LW13, SEQ ID NO: 36). The amino acid position at the start of the alignment is shown in parenthesis.

Figure 10: Amino acid alignment of Glutaredoxin subfamily 3 domain 2 across yeast and plant cDNA sequences (GRX5, SEQ ID NO: 10; AtQ9SV38, SEQ ID NO: 38; AtO80451, SEQ ID NO: 40; AtQ9LW13, SEQ ID NO: 36). The amino acid position at the start of the alignment is shown in parenthesis.

Figure 11: Amino acid alignments of yeast and plant cDNA sequences of glutaredoxin subfamily 4 showing the presence of two conserved domains. The abbreviations correspond to the "Gene ID" as explained in Table 6.

Figure 12: Amino acid alignment of glutaredoxin subfamily 4 domain 1 across yeast and plant cDNA sequences (AtQ9FVX1, SEQ ID NO: 30; EN1106C21909, SEQ ID NO: 18; AtQ9M457, SEQ ID NO: 32; EN1106C2582, SEQ ID NO: 22; OZ1116C12744, SEQ ID NO: 46). The amino acid position at the start of the alignment is shown in parenthesis.

Figure 13: Amino acid alignment of glutaredoxin subfamily 4 domain 2 across yeast and plant cDNA sequences (AtQ9FVX1, SEQ ID NO: 30; EN1106C21909, SEQ ID NO: 18; AtQ9M457, SEQ ID NO: 32; EN1106C2582, SEQ ID NO: 22; OZ1116C12744, SEQ ID NO: 46). The amino acid position at the start of the alignment is shown in parenthesis.

#### DETAILED DESCRIPTION OF THE INVENTION

In the specification at page 50, line 22, please replace the paragraphs which start with "YDR513w," with the following amended paragraphs:

YDR513w	primer	forward:
GGAATTCCAGCTGACCACCATGGAGACCAATTTTCCTTCGACT ( <u>SEQ ID NO: 51</u> )		
YDR513w	primer	reverse:
GATCCCCGGGAATTGCCATGCTATTGAAATACCGGCTTCAATATTT ( <u>SEQ ID NO: 52</u> )		

In the specification at page 51, line 2, please replace the paragraphs which start with "YER174c," with the following amended paragraphs:

YER174c	primer	forward:
GGAATTCCAGCTGACCACCATGACTGTGGTTGAAATAAAAAGCC ( <u>SEQ ID NO: 53</u> )		

YER174c

primer

reverse:

GATCCCCGGGAATTGCCATGTTACTGTAGAGCATGTTGGAAATATT (SEQ ID NO: 54)

In the specification at page 51, line 19, please replace the paragraph which starts with "5'-GGAATT," with the following amended paragraph:

5'-GGAATTCCAGCTGACCACCATGGCAATTCCCGGGGATC-3' (SEQ ID NO: 55)

In the specification at page 58, line 1, please replace the paragraph which starts with "The yeast and plant," with the following amended paragraph:

The yeast and plant cDNA sequences were translated into a predicted amino acid sequences and the relationship among the amino acid sequences was determined by sequence alignment and block alignment using the ClustalW algorithm in Vector NTI ver7. The glutaredoxin and thioredoxin genes were separated into four subfamilies based on this alignment as shown in Figure 1. The glutaredoxin family is characterized by the standard glutaredoxin domain defined in the Prosite database as an amino acid motif with the consensus sequence [LIVMD]-[FYSA]-x(4)-C-[PV]-[FYWH]-C-x(2)-[TAV]-x(2,3)-[LIV] [LIVMD]-[FYSA]-x(4)-C-[PV]-[FYWH]-C-x(2)-[TAV]-XX-[LIV] (SEQ ID NO:56) or [LIVMD]-[FYSA]-x(4)-C-[PV]-[FYWH]-C-x(2)-[TAV]-XXX-[LIV] (SEQ ID NO:57). Most sequences show the characteristic two cysteines that when reduced form either two thiol groups or when oxidized form a disulfide bond. Other proteins in this family have only a single C at this site.

In the specification at page 58, line 12, please replace the paragraph which starts with "Subfamily 1 contains," with the following amended paragraph:

Subfamily 1 contains the yeast genes GRX1 and GRX2 (figures 2-4). Domain 1 has the core sequence [VI]-[VF]-[VI]-X-[SA]-K-[TS]-[WY]-C-[PGS]-[YF]-[CS] (SEQ ID NO: 58). OZ1116C26232 and AtQ95K75 lack the C-X-X-C disulfide site and instead have a single C at this site. Domain 2 contains a motif defined as G-Q-X-T-V-P-N-[VI]-[FY]-[VI]-X-G-[KN]-H-I-G-G-[CN] (SEQ ID NO: 59).

In the specification at page 58, line 17, please replace the paragraph which starts with "Subfamily 2 contains," with the following amended paragraph:

Subfamily 2 contains both glutaredoxin GRX3 and GRX4 and thioredoxin THX1 and THX2 sequences (figures 5-7). This family has a region of homology comprising two domains.

In most sequences the domains are continuous, except in GRX3 and GRX4 in which the two domains are separated by two amino acids. Domain 1 has a core sequence of [VI]-V-[VL]-X-F-X-[TA]-X-W-[CA]-X-[PA]-[CS]-K (SEQ ID NO: 60). The region [CA]-X(2)-[CS] (SEQ ID NO: 61) contains C at position 1 or 4 or both. Domain 2 is a region of similarity that has a core sequence of F-X(2)-[VI]-[ED]-[AV]-[ED]-E-X(2)-[ED]-[IV] (SEQ ID NO: 62).

In the specification at page 58, line 25, please replace the paragraph which starts with "Subfamily 3 contains," with the following amended paragraph:

Subfamily 3 contains GRX5 and three plant sequences that have a single C amino acid at the putative active site (figures 8-11). The core sequence of domain 1 is V-[VM]-X(3)-K-G-X(4)-P-X-C-G-F-S (SEQ ID NO: 63). Domain 2 is defined by the sequence Q-[LI]-[FY]-[VI]-X-[GK]-E-[FL]-X-G-G-[CS]-D-[IV] (SEQ ID NO: 64).

In the specification at page 59, line 1, please replace the paragraph which starts with "Subfamily 4 contains," with the following amended paragraph:

Subfamily 4 does not have any members from yeast and is comprised of 5 plant sequences that have two domains of homology (figures 11-13). Domain 1 has a core sequence similar to subfamily 1 that is [VI]-V-I-F-S-K-S-Y-C-P-Y-C (SEQ ID NO: 65). Domain 2 has two regions with common sequences of V-V-E-L-D-X-R-E-D-G (SEQ ID NO: 66) and V-G-R-R-T-V-P-Q-V-F-[VI]-[NH]-G-K-H-[LI]-G-G-S-D-D (SEQ ID NO: 67).

In the specification at page 63, line 22, please replace the paragraph which starts with "Plants that exhibit," with the following amended paragraph:

Plants that exhibit tolerance of one abiotic stress often exhibit tolerance of another environmental stress or an oxygen free radical generating herbicide. This phenomenon of cross-tolerance is not understood at a mechanistic level (McKersie and Leshem, 1994, Stress and Stress Coping in Cultivated Plants, Kluwer Academic Publishers). Nonetheless, it is reasonable to expect that plants exhibiting enhanced drought tolerance due to the expression of a transgene might also exhibit tolerance of low temperatures, freezing, salt, air pollutants such as ozone, and other abiotic stresses. In support of this hypothesis, the expression of several genes are up or down-regulated by multiple abiotic stress factors including cold, salt, osmoticum, ABA, etc (e.g. Hong et al. (1992) Developmental and organ-specific expression of an ABA- and stress-induced

protein in barley. Plant Mol Biol 18: 663-674; Jagendorf and Takabe (2001) Inducers of glycinebetaine synthesis in barley. Plant Physiol 127: 1827-1835); Mizoguchi et al. (1996) A gene encoding a mitogen-activated protein kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 93: 765-769; Zhu (2001) Cell signaling under salt, water and cold stresses. Curr Opin Plant Biol 4: 401-406).

In the specification at pages 78-79, please delete the section entitled "Legend."